# Regulation of Tumour Necrosis Factor Receptor Expression on Neutrophils by Arachidonic Acid and Other Long Chain Fatty Acids.

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Thesis submitted for the degree of Doctor of Philosophy

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**June 2004**

**Summary**

Tumor necrosis factor (TNF) is a pro-inflammatory cytokine with multiple biological effects. The receptors for this cytokine on neutrophils have been shown to be rapidly down-regulated following activation, leading to the release of soluble forms of these receptors. Thus neutrophils become less responsive to TNF and the soluble TNF receptors (TNFR) serve to control TNF activity. During inflammation, leukocytes become activated as a result of the action of a variety of mediators. These mediators include not only cytokines but also lipids, such as the pro-inflammatory ω-6 fatty acid, arachidonic acid (AA) and its metabolites. Cellular activation leads to the release of AA from membrane phospholipids. AA regulates the function of many cell types including neutrophils. In view of the known pro-inflammatory properties of AA and the anti-inflammatory properties of ω-3 fatty acids, a study was undertaken to examine whether or not these fatty acids regulate the expression and release of TNFR in neutrophils.

While much emphasis has been placed on agonist-induced down-regulation of TNFR, our data show that AA causes a rapid (10-20 min) and dose-dependent (0.5 to 30 μM) increase (8-fold) in the surface expression of both classes of TNFR (TNFRI and TNFRII) on human neutrophils, at concentrations found in inflammatory fluids. This correlates with an increase in superoxide production to a TNF challenge. In contrast, both fMLP and LPS significantly reduce the expression of both TNF receptors. Interestingly, in neutrophils pretreated with AA, fMLP causes an increase in TNF receptor expression, consistent with AA preventing the fMLP-induced receptor release in neutrophil culture. In addition, while AA causes an increase in TNF receptor

expression on matured HL-60 cells (neutrophil-like cells), a decrease occurs on HUVEC and non-matured HL-60 cells. These data demonstrate a unique effect of AA on neutrophils.

The relationship between AA and the anti-inflammatory ω-3 fatty acids, DHA and eicosapentaenoic acid (EPA), in the modulation of TNF receptor expression has also been examined. These ω-3 polyunsaturated fatty acids, including linolenic acid (LNA), cause a decrease in TNFR expression on neutrophils. The ω-6 linoleic acid (LA) and ω-9 oleic acid (OA) both cause an increase in TNFR expression. Furthermore, pre-exposure of neutrophils to nanomolar amounts of EPA or DHA prevents the AA-induced up-regulation of TNFR. These results thus identify another mechanism of regulating the inflammatory reaction by the ω-3 fatty acids.

The mechanisms by which AA induces an increase in TNFR expression have been studied. Masking of the carboxyl group results in loss of activity. It is unlikely that a product of AA is responsible since neither the hydroperoxyeicosatetraenoic acid, nor hydroxyeicosatetraenoic acid derivatives show activity. Also, the effects of AA are not sensitive to the action of inhibitors of the cyclooxygenases and lipoxygenases. Using chemical inhibitors of intracellular signaling pathways, we demonstrate that the effect of AA on TNFRI is very sensitive to GF109203X, PD098059, AACOCF3 and wortmannin, showing a role for protein kinase C, the extracellular signal regulated protein kinases and cytoplasmic phospholipase $A_2$, and PI-3 kinase respectively, in the enhancement of TNF receptor expression by AA. Although the effects of AA on TNFRII are also decreased by the chemical inhibitors, the results show that these

signalling molecules only contribute in part to the mechanisms of increased TNFRII receptor expression.

The data presented in this thesis suggest a novel role for AA in the inflammatory reaction, through its action on neutrophil TNFR expression. The work has identified a unique effect of ω-3 polyunsaturated fatty acids for regulating this AA-induced increase in the expression of TNF receptors.

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